CONTRIBUTION TO THE BACTERIAL FLORA OF THE SYDNEY WATER SUPPLY, I.

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As far as I am aware, the only paper that has been published dealing with the bacterial flora of the Sydney water supply was contributed by Katz to these Proceedings in 1886. At that time bacteriology was beginning to perfect its methods of technique, and it is to be regretted that the five organisms in the paper are not sufficiently described to enable them to be diagnosed with any degree of certainty. It therefore seemed to the writer that a paper or a series of papers upon the bacterial flora of the Sydney water might be of interest to the Society.

The bacteria which will be hereafter described were obtained from the tap-water in the Society's Bacteriological Laboratory, which is supplied directly from one of the city reservoirs in Centennial Park, into which it is pumped from the central pumping station and reservoir in Crown Street. The water is originally taken from the Nepean, Cordeaux and Cataract rivers, the catchment area of which covers 345 square miles. From the rivers it is conducted by a series of tunnels and open canals to Prospect reservoir, thence by open canal and pipes to Pott's Hill reservoir, where it is screened through a series of fine sieves (840 meshes to the inch). From this reservoir it is led through iron pipes to Crown Street, whence it is pumped to the various city reservoirs. The water is not filtered, but care is taken to safeguard the purity of the supply by the rigid and continual inspection of settlements and stock within the catchment area and adjoining the reservoirs.

The object of this investigation was not merely to determine the bacteriological purity of the water but to discover the more commonly occurring micro-organisms. There are two methods generally employed in testing the purity of water. First, there is the method of counting, and this is the usual method because it is the easier. It, however, goes without saying that within wide limits the number of bacteria that are consumed in water is of far less importance than the kinds of bacteria, and especially if these kinds happen to be of an injurious nature.

The point wherein there is reason for the numerical test as indicating the purity or otherwise of water is that the numbers will be proportional to the food material, whether it be saline or organic, and in less degree to the temperature. It is obvious that the organic matter will represent inversely the degree of natural filtration to which the water has been subjected. Surface waters which have undergone little or no filtration will contain more organic matter than spring water which has had the bulk of the organic matter and micro-organisms removed by natural filtration through successive layers of soil and sand. However well a water may be naturally filtered, it is never sterile, and the organisms inherent in it will multiply to an extent proportionate to the food supply and to the temperature. Thus the number of organisms in the water may be taken to represent the organic matter, and therefore indirectly the degree of natural filtration. It is by natural filtration that the bacteria which inhabit the soil, be they noxious or otherwise, are removed, and in the majority of cases, especially where the gathering grounds are in populated localities, natural filtration is not considered to be sufficient, and so water is generally filtered through sand-filters before being supplied to towns.

Migula has formulated a scheme for obtaining an idea respecting the purity of water from the number of bacteria per cubic centimetre. Pure waters vary from zero to 1,000, mediocre from 1,000 to 10,000, and impure over this number. But he further remarked that of more importance than the absolute number of

bacteria are the number of species per cubic centimetre. In a pure water he considered that this should not exceed 10.

The media generally employed for growing the colonies are either meat-peptone-gelatine or meat-peptone-agar. In Sydney water many organisms rapidly liquefy the former and utterly prevent the appearance of the more slowly growing colonies. There are others that form amœboid colonies on the surface of agar, and rapidly spreading over the plate, obliterate the fixed colonies. In the beginning of my investigation it became evident that these media were unsuited for separating the bacteria; still in working with the agar media portions of the plates could sometimes be obtained in which the amœboid activity of the colonies had been restrained. Agar also throws up the colour of the surface colonies, and this is of some assistance in picking out species. The agar was improved to a certain extent by omitting, in the preparation of the medium, the peptone and the common salt and adding 2% dextrin or gum acacia which tends to restrain the amœboid growth of the colonies.

Hesse and Niedner employ an agar medium containing agar 1·25 grm. "Nährstoff Heyden" 0·75 grm., and water 98 c.c. Abba advises a medium containing Liebig's meat extract 6 grms., gelatine 150 grms. and distilled water 1000 c.c. This is after solution made neutral to phenolphthalein and rendered alkaline by the addition of 0·5 grm. anhydrous sodium carbonate. The plates are incubated for 10 days at 18-19° C. He claims that more colonies develop in this medium than when an agar medium (such as Hesse and Niedner's) is employed. I have confirmed this, but I have also found that the bacteria of Sydney water grow so freely in this medium that the colonies have in four days so liquefied the gelatine that observation under the microscope is impossible, and that the plate is almost entirely liquefied in six days. A temperature of 15° C. is more suitable for separating bacteria, but even at this temperature the colonies cannot be allowed to grow for longer than four days. With 30 colonies per plate liquefaction had proceeded so fast in six days that separation was impossible.

In separating the bacteria both Abba's gelatine and dextrin-meat agar were employed. The latter medium is prepared by dissolving 20 grms. of agar in 1000 c.c. of ordinary meat extract in the autoclave. After clarification with white of egg, 10 c.c. of the mixture are pipetted into warm water and neutralised to phenolphthalein with tenth-normal sodium hydrate. The calculated quantity of normal sodium hydrate is added to the bulk, together with 0·5 grm sodium carbonate; 20 grms. of dextrin or gum acacia, dissolved in a small quantity of water is added, and the medium boiled, filtered, placed in test tubes and sterilised. The water was allowed to flow from the tap for half-an-hour upon a sterilised watch-glass supported upon a tripod. From the watch-glass the water was taken up into a sterile graduated pipette and added to the previously melted and cooled gelatine (30° C.) or agar (42° C.); the tube was then shaken and the contents poured into Petri dishes, which, after setting, were inverted and incubated at the requisite temperature (15°, 18° or 22°). When the colonies had grown sufficiently, inoculations were made upon sloped agar and in gelatine (stab). When a diagnosis could not be made from these—and this was the case when the organisms were obtained for the first time—a series of gelatine plates were prepared. The colonies that developed on these plates were used both for the diagnosis of the organism and for obtaining a pure culture. Generally the cultures obtained from the primary (water) plates were impure.

Having obtained a pure culture of an organism, inoculations were made upon the ordinary media, which included peptone-meat-agar, peptone-meat-gelatine, peptone-meat-bouillon, glucose-peptone-meat-gelatine, lactose-peptone-meat-gelatine peptone-meat-bouillon with 0·5% nitrate of potassium, ordinary potato, and ordinary defatted milk. With the exception of the potato and the milk, all the media were neutralised with sodium hydrate to phenolphthalein as has been described. In observing the motility, a portion of a young agar culture was transferred to a drop of normal saline and examined. If the organisms appeared non-

motile a further observation was made upon a young bouillon culture.

The difficulties of diagnosing bacteria in the absence of typical cultures is admitted, and in the descriptions which follow the points which led to the identification of the organisms are noted. Cultural characters which have not been previously published, are also given. One of the points in which I have found a difference is the size and especially the breadth of the organism. Bacteria differ in dimensions slightly, according to the medium in which they are grown. The sizes which I have given are of the bacteria when cultivated upon agar, and they have been measured by a method which I shall describe in a future paper. In describing the colours or shades of colour, I have used as a reference the tints upon a sample card of enamel paints issued by the firm of Blundell, Spence and Co., London.

With a temperature of 15° C., I have found an average of about 100 bacteria in the laboratory tap-water during the months of May and June. The organisms are not at all evenly distributed in the water as it issues from the tap. For example, in five plates inoculated with 0·1 c.c. there developed upon Abba's gelatine 15, 15, 12, 5 and 4 colonies; this is equal to an average of 100 bacteria per cubic centimetre.

Micrococcus candidus, Cohn.

The organism measures from 0·5 to 0·6 μ in diameter, and occurs singly, in twos and in groups. It stains deeply with methylene-blue. On agar, there is formed a broadening, translucent white, raised, moist glistening stroke. The iridescent and white surface colonies, on agar plate, are seen, under a sixty-fold magnification, to be finely granular, and to have a clear, lacerate margin. In gelatine stab culture the growth is filiform, with a flat, spreading, white nail-head.

Sarcina lutea, Schröter.

This organism forms a narrow, moist glistening, convex, canary-coloured ridge in agar stroke culture. The individuals

measure about 1 μ, and when taken from cultures on solid media are found in sets of two and four. In fluid culture they occur in badly defined packets. There is a filiform growth with a raised nail-head in gelatine stab culture, and on gelatine plates the colonies are circular and, when magnified, appear finely granular and smooth-edged.

Bacterium album.

Bacillus albus, Eisenberg.

On agar stroke there is formed a porcelain-white, narrow, convex layer with a smooth edge. The consistency is firm or leathery, and there is produced a strong latrine odour. The bacterium is a rod with rounded ends, is actively motile and measures 0·4 : 1-2 μ. It does not stain when treated by Gram's method. The colonies on agar are porcelain-white and raised. In gelatine the surface colonies are irregular and white. Under a moderate power the margin is seen to be lacerate-erose, the centre marbled (*B. coli commune* type). The deep colonies are circular, zonate, and have a smooth edge. In gelatine stab culture the needle track is filiform; at the top there is a white, flat nail-head which spreads irregularly. The upper part of the stroke becomes tuberculate. No gas is produced in glucose-gelatine, and milk is not coagulated. Bouillon becomes very turbid, and a white precipitate and surface ring is formed. The indol reaction was not obtained. The growth on potato is white, moist glistening, and spreads over the surface; it ultimately becomes stone-coloured. The organism grows well at 22° C., but not at 37° C.

Eisenberg describes the potato culture as restricted to the place inoculated.

Bacterium sinuosum, Wright.

On the agar slope there is formed a thin, translucent white layer from which amœboid processes extend and cover the agar surface. The growth on gelatine is rather thin; the colonies

appear as translucent white, raised spots, which when magnified are seen to have a wavy, erose margin and a transparent crumpled centre. Gelatine is not liquefied. In stab culture there is formed a terraced nail-head. The organism is small and oval, actively motile, and measures 0·3 : 0·6 μ. It is decolorised when treated by Gram's method. Gas is produced from glucose, and milk is not coagulated. Bouillon becomes turbid, and forms a flocculent precipitate. A slight indol reaction is obtained. On potato the growth is moist glistening, flat, spreading, and of a light stone colour, which deepens to a light drab.

This organism is decidedly small, while Wright describes it as medium-sized. The growth on potato shows a difference from *Bact. minutum*.

Bacterium No. 46, Conn.

This is a short rod-shaped bacterium with rounded ends, and measures 0·4 : 1-1·5 μ. It is actively motile, and is not stained by Gram's method. On agar it forms circular white colonies, and on gelatine translucent white colonies that sink into the medium. When the gelatine colonies are viewed under a moderate power, they are seen in the deep to be rounded or irregular, almost opaque and apparently flocose. The surface colonies are circular with fluid contents in which there are large brown floccules. On agar stroke there is formed a pale white, moist glistening, flat expansion. In gelatine stab culture the liquefaction is crateriform, then saccate and tubular. There is a uniform turbidity, and no film forms on the surface. After a month the liquefied gelatine is yellow and turbid near the surface, clear below, and at the bottom of the tube there is a yellowish-white granular precipitate. On potato the dry glistening, brownish layer which is first formed becomes yellowish-brown, shining, flat and irregular. The potato is darkened. No gas is produced in glucose-gelatine. Nitrates are reduced to nitrites. Milk is coagulated, the reaction being acid.

Conn describes the agar stroke culture as white, becoming yellowish, and the reaction of the milk when coagulated as

alkaline. Otherwise they appear to be the same organisms. The difference is perhaps too slight to justify this being called a new species.

Bacterium gasoformans, Eisenberg.

Bubbles of gas are formed in the depth of the ordinary solid media. The cells are oval or sausage-shaped, and measure 0·5 : 1-2 μ; they are actively motile. The colonies, on gelatine plate, are circular and translucent white. Under a sixty-fold magnification the surface colonies appear rounded with a slightly erose margin; the centre is brownish and finely granular. The contents of the colony appear in motion. At a later period the contents become flocculent and the margin diffuse. In gelatine stab culture the liquefaction is crateriform, then stratiform and tubular. The stroke on agar is dirty-white, moist glistening and at the base spreading Gas bubbles are formed in the agar. On potato there is a scanty growth which is almost indistinguishable from the medium. Milk is coagulated, the reaction being acid. Nitrates are reduced to nitrites.

Bacterium aurescens, Ravenal.

This motile organism is oval, and measures 0·3-0·4 : 1 μ. It forms threads in old cultures. In agar stroke culture the first growth is a thin yellowish-white line, which as it broadens becomes primrose-coloured, and ultimately of an old-gold shade. In gelatine stab culture there is a filiform growth; the gelatine at the upper part of the stab becomes slightly consumed, and eventually the growth around the consumed portion becomes tuberculate and yellowish-white. The colonies on gelatine are yellowish, and slowly liquefy the gelatine. Under a sixty-fold magnification they are seen to have a lacerate, transparent margin, and a pale yellowish-brown, marbled or convoluted centre. The older surface colonies have the central convolutions more marked, an annulus with coarse flakes floating in the liquefied medium, and a ciliate margin. The deep colonies are brown and rounded.

On potato the growth is orange-yellow, flat and glistening. No gas is developed in the presence of glucose, no indol is produced in bouillon, and milk is not coagulated.

The colonies on gelatine appear to differ from those described by Ravenal, but as the other characters agree this may be a variety.

Bacterium pullulans, Wright.

This is a short rod with rounded ends, and measures 0·3:0·6-1 μ; it is actively motile. On agar the colony is deep yellow and circular, with a smooth edge; the stroke is deep yellow or old-gold in colour, convex, moist glistening and restricted. The gelatine stab is at first filiform with a spreading deep yellow nail-head. The gelatine becomes slowly liquefied; the liquefied area is funicular and turbid; a deep yellow precipitate is formed. In gelatine plate culture the surface colonies have a deep yellow centre and a pale yellow margin. When magnified sixty-fold they are seen to have an irregular zonate centre and lobulated margin, from the under-surface of which rounded buds are given off. The small colonies, which have budded off, lie free around the lobular margin, and are either circular or irregular, according to their size, and brown in colour. Milk is not coagulated, and a trace of indol is produced in bouillon culture.

Bacterium cuticulare.

Bacillus cuticularis, Tils.

A rod with rounded or slightly pointed ends, measuring 0·4 : 0·8-1 μ. It is motile and is stained by Gram's method. In gelatine plate culture the deep colonies are, when magnified, seen to be circular, brownish and zonate. The surface colonies are transparent and crumpled or contoured with an irregular, erose margin like *Bact. coli commune*. The stroke on agar is at first pale white and iridescent, but becomes moist glistening and of a primrose colour; when touched with the needle the culture has a viscous consistency. The stab in gelatine is faintly filiform, but

oon becomes shallow crateriform. There is practically no growth in the depth of the medium, and a primrose-coloured film lies on the liquefied gelatine. There is no gas developed from glucose, and milk is coagulated, the reaction being neutral. On potato the growth is canary-coloured, but changes to a pale yellow; it is raised and irregularly spread. Nitrates are not reduced. In bouillon a turbidity is formed; there is a precipitate, but no film. A strong indol reaction was obtained.

Bacterium arborescens.

Bacillus arborescens, Frankland.

On gelatine the colonies are pale yellow and circular, with a slight mycelioid appearance. The medium is liquefied round the colony. When magnified the deep colonies are seen to have an irregular centre, from which root-like fibres extend; these are closely packed together and yellowish. The surface colonies have an indefinite centre, from which loosely twisted and sharply bent comparatively wide strands apparently anastomose. The stab in gelatine is filiform, with a smooth, moist glistening, deep yellow nail-head. The gelatine is slowly liquefied in a crateriform manner, and just below the liquefied medium the filiform stab becomes expanded and diffuse. The colonies on agar are circular, raised, moist glistening and of a translucent pale buff colour. When magnified they are seen to have a granular centre, with a transparent irregular margin, beset with short irregular processes. The deep colonies are rounded, oval or lenticular and rough. The agar stroke is moist glistening, raised and spreading. The colour is a deep yellow which changes, especially in the centre of the growth, to old-gold. No gas is formed in glucose-gelatine, and nitrate is not reduced. Bouillon becomes turbid, and there is a filamentous precipitate, but no film. A slight indol reaction was obtained. Milk is not coagulated. On potato the growth is flat, spreading, glistening and deep yellow in colour. The organisms are rods with rounded ends, and may be long or short; the average size is $0{\cdot}3 : 2\ \mu$.

Bacterium miniaceum.

Bacillus miniaceus, Zimmermann.

On agar slope a brilliant vermilion, raised, spreading, moist glistening stroke is formed, from the bottom of which amœboid processes spread out and gradually cover the entire lower surface. The organism is a cocco-bacterium measuring 0·7 : 1 μ. In gelatine plate culture the colony quickly liquefies the gelatine, forming a crateriform, pink area. In gelatine stab the medium is liquefied in a stratiform manner, the fluid being very turbid from floating pink granules. The red pigment is not bleached by zinc and hydrochloric acid.

Bacterium rubefaciens.

Bacillus rubefaciens, Zimmermann.

On gelatine plate at 15° C., circular, raised, moist glistening colonies of a pale coral colour are formed. The medium is not liquefied. Under a sixty-fold magnification the surface colonies are seen to be rounded, reddish-brown and with a smooth edge; the contents are finely granular. The deep colonies are irregular and opaque. The bacteria are motile rods with rounded ends, and measure 0·45 : 1-1·5 μ; they occur singly and in twos. They are decolorised by Gram's method of staining. The agar stroke is raised, glistening and restricted, the edge slightly puckered. At 22° C. the colour is light oak, while at 15° C. it is of a pale terra-cotta colour, which eventually becomes pale coral. On potato at 22° C. there is formed a light oak, scanty, glistening growth; the medium in the vicinity of the growth is darkened. At 15° C. the colour is like the agar culture, pale terra-cotta, and when now incubated at 22° changes to a watery, raised, light orange growth; the potato beneath becoming brownish. Bouillon becomes slightly turbid; there is no film formed, and a compact, pale coral precipitate is produced. The gelatine stab is filiform with an irregular, flat, slightly terraced nail-head; the colour is either pale coral or light oak, according to the incubation-

temperature. Milk is not coagulated, nitrate is not reduced, but a trace of indol is formed in bouillon.

Zimmermann describes the organism as forming a blue-grey stroke on agar.

Bacterium salmoneum, Dyar.

The organism grows scantily in artificial media. On gelatine plate the colonies are small, hemispherical, moist glistening, and of a pale scarlet colour; when magnified the structure is seen to be finely granular and the margin smooth. The deep colonies are rounded and opaque. The stab in gelatine becomes filiform, and bears a small, rounded and raised vermilion nail-head. On potato there is practically no growth. Bouillon becomes turbid; there is a precipitate and a light reddish-coloured film. No indol is formed. On agar the stroke is narrow and restricted, at first coral-pink, then becoming light orange, and ultimately reddish-orange. The organisms are non-motile thin rods with rounded or slightly pointed ends; they stain irregularly, and measure generally $0{\cdot}4 : 2\ \mu$. Milk is not coagulated, and the reaction is unchanged. Nitrates are reduced to nitrites.

Bacterium janthinum, Zopf.

When grown upon agar the organism forms a deep violet, moist glistening, irregularly raised layer with a white margin and smooth edge; the consistency is gelatinous. The colonies in gelatine appear as very pale violet indefinite areas, the parent colony having sent out into the gelatine processes which have formed sub-colonies. Both the indefinite parent colony and the older sub-colonies are beset with processes similar to those of *Bact. Zophii.* The younger colonies have a moruloid centre, and are surrounded by circular or irregular sub-colonies similar in appearance to the colonies of *Bact. pullulans.* The stab in gelatine scarcely grows in the deep; the film is violet-coloured and spreads over the surface, sending down into the medium hair-like processes which appear like a hanging veil. The

gelatine begins to liquefy in two weeks, the liquefaction being shallow-stratiform. The organism is a motile rod with rounded or pointed ends, and measures 0·4-0·6 : 1-1·5 μ; involution forms in the shape of clubbed threads are soon found in the cultures. On potato there is formed a deep violet, irregularly spreading, flat, glistening layer. Bouillon becomes turbid with a flocculent precipitate and a strong violet film. The fluid becomes colourless and then violet. The indol reaction was obtained in the fluid after the elimination of the suspended bacteria by means of calcium chloride and ammonia. A control test without nitrite was made at the same time. A strong blue film formed on milk which was not coagulated; the reaction was alkaline.

Bacterium janthinum II., n.subsp.

The stroke on agar is violet, moist glistening, and slightly raised, the margin is beset with processes which are at first white but soon become like the centre of the stroke; the consistency when the culture is touched with the needle is found to be thin. The colonies in gelatine plate are circular, crateriform, pale violet and zonate; when magnified the contents are seen to consist of large granules; the edge is diffuse. In gelatine stab the liquefaction of the medium is rapid. The liquefied area is funicular, there is a violet deposit and a thin film of the same colour; the liquefied medium is turbid. A slight film is formed on bouillon, but there is no film formed on milk, which becomes faintly acid in reaction. In other respects the organism is identical with that described above.

The main points of difference between this subspecies and Zopf's organism are the rapid liquefaction of the gelatine (24 hours), the diffuse growth on agar and the absence of an alkaline reaction in milk.

Bacterium putidum, Flügge.

On agar slope there is quickly produced a luxuriant, moist glistening, raised, white layer which spreads over the lower

portion of the medium. The latter becomes strongly greenish fluorescent. The organisms are oval and measure 0·4 : 1-1·5 μ. They are actively motile. The stab in gelatine is filiform with a flat, irregular, moist glistening, yellowish-white nail-head. The upper part of the stab becomes tuberculate. No fluorescence of the gelatine was observed. Milk was not coagulated and the reaction remained neutral.

Bacterium fluorescens mutabile, Wright.

i.—This is a rod-shaped bacterium with rounded ends measuring 0·5 : 0·8-1 μ and is not coloured by Gram's method of staining. It is actively motile. The stroke on agar is greenish-white and moist glistening; the growth is luxuriant and quickly widens to the sides of the glass at the bottom of the stroke. The agar becomes strongly fluorescent. The gelatine in stab culture becomes liquefied in a funicular manner : the liquefied portion is turbid with floating floccules, and there is a yellowish-white precipitate and film. There is no fluorescence of the medium. Milk is coagulated and has an alkaline reaction. On potato, a moist glistening, drab-coloured expansion covers the surface of the medium.

ii.—This organism is a trifle larger than the above and measures 0·6 : 1-1·5 μ. The stroke on agar is drier and whiter than i., that is, it is less translucent. It is also less luxuriant in its growth and not so strongly fluorescent. Otherwise the characteristics are similar.

The two organisms are closely allied to *Bact. pyocyaneum*, differing mainly in fluorescence. They are best described by Wright's designation, and one of them is probably identical with his organism.

Bacillus subtilis, Ehrenberg.

The hay bacillus forms on agar slope a dull flat membranous layer with an irregular lacerate and ciliate margin. The membrane quickly spreads over the greater part of the agar surface.

When grown at 22° C. the lower part of the growth may or may not become slightly wrinkled or folded. On potato there is formed a dry, flat, white, mealy, spreading layer which soon becomes slightly speckled. The organism is an actively motile rod with rounded ends and measures 0·8 : 2-3 μ; it forms oval, central spores. The colonies on gelatine and agar are flocose. In gelatine stab culture the growth is very rapid; the medium is liquefied in a funicular or tubular manner, but soon becomes stratiform. The liquefied gelatine, which is at first strongly turbid, becomes clearer, while a strong white film forms on the surface; the sediment is white and flocculent.

Bacillus mycoides, Flügge.

As its name implies, this organism in gelatine and agar culture forms colonies that appear mycelioid. The mycelial processes grow over the surface and penetrate into the medium. It is a rod-shaped bacillus with rounded ends, and occurs singly and in threads; the individuals measure 1 : 3 μ; oval spores are formed in the middle of the rod. According to Flügge it is non-motile, and to Zimmermann it is motile. The individuals separated from the Sydney water supply are motile. The track of the needle in gelatine stab culture becomes filiform, then villous, and finally arborescent. The gelatine is liquefied slowly, and becomes stratiform with a strong white film.

Bacillus ramosus liquefaciens, Flügge.

The colonies on the gelatine plate are mycelioid and white. When magnified the deep colonies are seen to be fibrous and nodular, the surface colonies to have an irregular dark centre from which root-like fibres studded with minute nodules extend. The gelatine is softened round the colony. The stab in gelatine is filiform, but soon becomes arborescent; there is slowly formed a dry, white, irregularly depressed and spreading nail-head from which branches are sent downwards into the medium. There is no liquefaction apparent after three weeks at 15° C. It is a thin

rod with rounded ends and occurs singly and in chains. The individuals measure 0·6 : 2-3 μ and form oval, central spores. It is stained by Gram's method, and the single cells which have been grown in bouillon at 37° C. are motile. On agar at 22° there is formed a thin, white, spreading, syrupy layer. At 37° a brownish-white, flat layer rapidly spreads over the agar-surface, which soon appears as if it had been dusted with oatmeal. The upper portion of the growth is furrowed laterally and downwards. The condensed water has a strong crumpled skin. It has little similarity to *Bac. mycoides*. On potato a dry, crumpled, thin layer spreads over the surface. The colour is at first white with a fawn tinge, later it becomes reddish-brown; the potato becomes brownish. Bouillon is rendered turbid; a precipitate and a strong crumpled whitish film are formed. The indol reaction was not obtained. Milk becomes alkaline in reaction and is coagulated when warmed. Nitrates are slightly reduced to nitrites.

Frankland describes a *Bac. ramosus* which agrees with this organism fairly well except in size; he quotes it as measuring 1·7 : 7 μ. Flügge describes his bacillus as moderately large. *Bac. implexus*, Zimm., liquefies gelatine much more quickly than this organism and it measures 1·15 : 2·5 μ.

Bacillus gracilis, Zimmermann.

This is an aerobic bacillus with a terminal spore. The young rods are actively motile. In gelatine the colonies appear as dry, white specks which do not liquefy the medium. Under a sixty-fold magnification the deep colonies appear circular and opaque. The amœboid surface colonies appear very granular and grained. The gelatine stab is filiform with a small nail-head, which becomes moist glistening and amœboid, while the filiform track of the needle becomes tuberculate near the top. The stroke on agar is narrow, white, moist glistening and raised; the margin is ciliate. It does not appear to grow on potato. The bacilli are long rods with rounded ends and measure 0·3-0·4 : 3-8 μ.

Bacillus circulans, Jordan.

The colonies, in gelatine, are white and sink into the medium, forming a cylindrical pit or hole which widens out at the top when the colony has grown as far as it can downwards. The medium is consumed and not liquefied. With a sixty-fold magnification the dark granular contents of the colony are seen to be in active motion, apparently circulating round the walls of the pit. The deep colonies are at first circular, irregular or amœboid, but the latter soon become round and rapidly open to the surface. In gelatine the stab assumes the form of a hollow inverted cone. No gas is produced in glucose-gelatine. The organism is a rod with rounded ends and forms a terminal round spore which becomes 1 μ in diameter. The vegetative forms measure 0·5 : 2-3 μ and are actively motile. They retain the colour when treated by Gram's method. Both on agar plate and agar slope there is formed an amœboid growth which rapidly spreads over the entire surface. In milk the casein is slowly peptonised without coagulation. Bouillon becomes turbid and there is formed a white film and a flocculent and filamentous precipitate. No indol is formed and nitrate is not reduced. A drab-coloured growth spreads over the surface of potato.

Cladothrix dichotoma, Cohn.

On gelatine and agar media, the colonies grow as scattered white points which become circular, porcelain-white and raised. The larger colonies develop a marginal ring within which are little central crusts. A deep brown colour is diffused into the media. Gelatine is not liquefied. The fungus appears microscopically as a narrow branching mycelium, 0·4 μ in breadth, and broken up into short rods and threads. The colonies on agar appear, when magnified, circular and opaque with a ciliate margin.

Bacterium album mesentericum, n.sp.

Shape, etc.—A slender rod with rounded ends, averaging 0·3 : 1·5 μ. It is slowly motile and is decolourised by Gram's

method of staining. No spores were observed. It grows well at 37°.

Agar plate.—Circular, white, moist glistening, raised colonies are formed. When magnified they are seen to have a smooth edge; the internal structure is slightly marbled. The deep colonies are irregular and rough.

Agar stroke.—A luxuriant, white, raised layer with smooth iridescent edge grows along the stroke. The edge becomes lacerate.

Gelatine plate.—White, circular liquefied areas are produced. Under a sixty-fold magnification, the subsurface colonies are seen to be irregular, dark brown and apparently flocose, the surface colonies have a flocose centre and a clear margin.

Gelatine stab.—At first the stab is slightly crateriform but becomes funicular. The liquefied gelatine becomes turbid and a white precipitate forms. No film appears and the turbidity persists.

Glucose-gelatine.—No gas is produced.

Bouillon.—The medium becomes turbid, a slight film is formed as well as a pale white precipitate. Traces of indol are produced.

Milk.—The casein is coagulated and the reaction becomes acid.

Potato.—There is formed at first a pale ochre moist glistening, raised growth, which becomes yellowish-brown, irregular and greasy. The layer continues to rise from the surface of the potato, and when growth stops it is mesenteric, dull, and of a light drab colour. The potato is darkened.

Nitrate-bouillon.—The nitrate is reduced to nitrite.

The affinities of this organism are with *Bact. Fairmontensis*, Wright, *Bac. aquatilis communis*, and *Bact. No. 46*, Conn. From the first named it differs in the appearance of the gelatine colonies and the growth in gelatine stab culture as well as in the growth upon potato. *Bac. aquatilis communis* is considered by Kruse to be a *Bact. fluorescens liquefaciens* which has lost its power of producing fluorescence. It therefore differs from this organism in the reaction of the milk when coagulated. *Bac. aquatilis communis* forms a yellowish-brown or reddish layer on potato and is never

mesenteric. The liquefied gelatine in stab culture is at first turbid but soon clears, and further it is a medium-sized rod, 0·6 : 1·2-2·5 μ, and very motile. *Bact. No. 46*, Conn, measures 0·4 : 0·8 μ, and forms a yellowish or brownish (presumably flat) layer on potato; its colour upon agar and gelatine is yellowish-white and it coagulates milk, the reaction being alkaline.

Bacterium aerofaciens, n.sp.

Shape, etc.—This is an oval bacterium measuring 0·4 : 0·8-1 μ. It is motile and is not stained by Gram's method. It grows well at 37° C. and at 22° C. No spores were observed.

Agar plate.—There are formed translucent white, wax-like, circular, raised colonies, which when magnified are seen to be homogeneous, circular and smooth-edged. The deep colonies are irregular and rough.

Agar stroke.—The growth is raised, moist glistening, grey-white, and spreads irregularly from the inoculating line.

Gelatine plate.—The colonies are at first translucent white and slightly irregular, later they become amœboid and iridescent. When magnified the surface colonies are seen to be yellowish-brown and finely granular with a lacerate-erose margin; the deep colonies are brown or opaque and rounded with a smooth edge.

Gelatine stab.—The line of inoculation becomes filiform, with a translucent white nail-head, which spreads out and becomes somewhat depressed. Gas bubbles appear in the depth of the medium.

Glucose-gelatine.—Gas is produced in quantity.

Bouillon.—The medium becomes turbid, a precipitate is formed, but no film appears. No indol reaction was obtained.

Nitrate-bouillon.—Nitrate is strongly reduced to nitrite.

Milk.—The medium is not coagulated.

Potato.—A white, moist glistening, flat growth, which covers the surface of the medium, is formed, and eventually it becomes the same colour as the potato.

The characters of this organism show that it has its closest allies in the hog-cholera (*Bact. suipestifer*) class of bacteria. The

production of gas in ordinary nutrient gelatine marks it as being new. Its closest ally appears to be *Bact. sinuosum.*

BACTERIUM MINUTUM, n.sp.

Shape, etc.—A small cocco-bacterium measuring 0·4 : 0·5-0·7 μ. It is actively motile and is not stained by Gram's method. No spores were observed. It grows well at 37°.

Agar plate.—A translucent white, amœboid colony quickly grows over the surface. The amœboid processes are narrow and radiate from a central point. The structure is homogeneous and the margin smooth.

Agar stroke.—A white, moist glistening, raised growth becomes amœboid at the base. It may produce gas bubbles in the medium.

Gelatine plate.—The colonies are white and of the *B. coli* type. When magnified the deep colonies are irregular or rounded and either dark brown or opaque. The surface colonies are transparent yellowish, grained or contoured, and with an erose, rounded margin.

Gelatine stab.—The stab becomes filiform and there is formed a flat, irregularly spreading nail-head. The surface growth becomes glistening, slightly depressed and contoured. Some specimens produce gas bubbles in the medium.

Glucose-gelatine.—There is a considerable development of gas.

Bouillon.—The medium becomes turbid and there is formed a filamentous precipitate but no film. There is no indol produced.

Nitrate-bouillon.—Nitrate is strongly reduced to nitrite.

Milk.—The medium is not coagulated in the cold. The reaction is acid and coagulation occurs on warming.

Potato.—There is formed an irregular, canary-coloured growth, sharply raised from the medium, which becomes dark and bluish in colour.

The organism was frequently found in the water. It has its allies in the *B. coli* and *suipestifer* groups. The potato growth is characteristic.

Bacterium croceum, n.sp.

Shape, etc.—An actively motile rod with rounded ends, measuring 0·5-0·6 : 2-3 μ; it occurs singly, in pairs, chains and threads. It is stained by Gram's method. The organism grows best at 22° C. No spores were observed. It grows well at 37°.

Agar plate.—The colonies are circular, raised, moist glistening and buff-coloured. When magnified they are seen to have a smooth, sinuous edge and a folded or grained internal structure. The deep colonies are irregular, moruloid and slightly ciliate.

Agar stroke.—There is formed a deep yellow, moist glistening, luxuriant, spreading and slightly raised layer. The edge is at first lacerate, but becomes smooth and the colour deepens to a light orange.

Gelatine plate.—The colonies are pale buff and rounded within a zone of softened gelatine. The surface colonies when magnified (60 times) are seen to have a brownish centre and a colourless, irregularly lobed margin, within which the colony appears crumpled. The deep colonies are zonate, the zones being coloured different shades of brown; the marginal zone appears greenish.

Gelatine stab.—The growth in the deep is very slight. The surface growth is deep yellow, depressed and restricted; the medium in the neighbourhood of the film is softened. The upper part of the stab becomes, after 14 days, faintly tuberculate.

Glucose-gelatine.—No gas is produced.

Bouillon.—The medium becomes turbid with floating floccules and there is formed a scanty, white, filamentous precipitate. There is no film produced and no indol formed.

Nitrate-bouillon.—There is no growth in this medium.

Milk.—The medium is unaffected.

Potato.—There is no growth either at 15°, 22° or 30° C.

The absence of growth upon potato, the brilliant deep yellow colour upon agar and the slow liquefaction of gelatine media separate this organism from all others that have been previously described. The nearest ally is perhaps the non-motile *Bac. fuscus liquefaciens*, Dyar.

BACTERIUM PALAEFORMANS, n.sp.

Shape, etc.—A rod-shaped bacterium with rounded ends, measures 0·5-0·6 : 1·5-2 μ and occurs singly, in chains and threads. It is actively motile and is stained by Gram's method. It does not form spores. There is little growth at 37°.

Agar plate.—The colonies are circular, pale buff, raised and moist glistening. When magnified the structure appears finely granular and the margin erose. The deep colonies are irregular, brownish and rough.

Agar stroke.—A narrow, primrose-coloured, moist glistening, raised stroke spreads out sharply at the base, when the condensed water has been reached, until the sides of the tube are touched, thus giving the culture the appearance of a spade. The colour slowly deepens to buff. The consistency is found to be thin when the culture is touched with a needle.

Gelatine plate.—The colonies are zonate, pale buff in colour, and have sunk into the gelatine, which is slightly liquefied. When magnified sixty-fold, the deep colonies are seen to be circular, brownish and annular; the surface colonies are either circular with a corrugated dark border or lacerate-erose with a coarsely granular centre.

Gelatine stab.—There is no growth in the depth of the medium. The gelatine is liquefied in a crateriform and afterwards in a stratiform manner. The scanty deposit is deep yellow and like a crumpled film.

Glucose-gelatine.—There is no gas produced.

Milk.—The medium is not coagulated.

Bouillon.—The medium becomes slowly turbid and there is formed a thin, pale yellow film and a scanty, filamentous precipitate. The indol reaction was not obtained.

Nitrate-bouillon.—Nitrate is not reduced.

Potato.—No growth was obtained either at 22° or 30° C.

This appears to be a motile form of *Bact. dormitator*, Wright, but perhaps its closest ally is *Bact. croceum*, from which it differs in colour, luxuriance of growth, and character of the gelatine

colonies. It can withstand limited amounts of alkali, since it was obtained upon agar containing 0·5 % sodium carbonate.

Bacterium subflavum teres, n.subsp.

Shape, etc.—The organisms measure 0·6 : 1·5 μ. Shorter and longer forms occur. The cells are oval and are actively motile. They are not stained by Gram's method. No spores were observed. It grows well at 37°.

Agar plate.—The colonies are circular, raised, moist glistening and buff-coloured. When magnified, the structure appears granular and the edge smooth. The deep colonies are opaque, irregular and rough.

Agar slope.—The growth is moist glistening, buff-coloured, raised and viscous; it spreads out at the base and the colour becomes deep yellow.

Gelatine plate.—The *ærogenes*-like colonies are cream-coloured and circular, moist glistening and slightly raised. When magnified, the deep colonies are seen to be circular and zonate; the surface colonies are circular, finely granular and brownish-yellow in colour.

Gelatine stab.—A scanty filiform growth is crowned by a raised nail-head, which spreads regularly. The convex growth is light buff and moist glistening.

Glucose-gelatine.—No gas is developed.

Bouillon.—The medium becomes slightly turbid and there is formed a filamentous precipitate and slight film. A slight indol reaction was obtained.

Nitrate-bouillon.—Nitrate is not reduced.

Milk.—The reaction becomes alkaline and there is no coagulation even upon warming.

Potato.—A canary-coloured, moist glistening, spreading, flat layer is formed.

The organism grows moderately quickly, and in this respect differs from its near allies *Bac. aureus*, Adametz, and *Bac. aurantiacus*, Frankland. The *ærogenes* type of the gelatine colonies

separates it from the *coli* type of *Bac. aureus* and *Bac. subflavus*, Zimmermann. Its nearest ally appears to be Zimmermann's organism, of which it is probably a variety.

BACTERIUM ARBORESCENS AMETHYSTINUM, n.subsp.

Shape, etc.—It is a very thin rod with rounded ends, and measures 0·2-0·3 : 1·5-2·3 μ. It is not motile and is not stained by Gram's method. Threads are formed in bouillon. No spores were observed. It grows best at 22° and 30°; there is little growth at 37°.

Agar plate.—The colonies are thin and diffused, of a blue white colour and translucent. When magnified sixty-fold, the centre is seen to be granular and the margin indefinite with watery transparent processes extending outwards. The deep colonies are rounded or oval, and slightly moruloid.

Agar slope.—A translucent, moist glistening, white growth, with a decided violet tinge. The margin is indefinite and sends off watery irregular processes which have a greenish colour. The growth is luxuriant and soon spreads to the sides of the tube. The condensed water becomes pinkish-cream coloured.

Gelatine plate.—The colonies appear like delicate moulds. When magnified the structure is seen to be mycelioid or root-like. There are prominent thick main trunks, and between these delicate radial threads fill up the spaces and give the colonies a circular shape.

Gelatine stab.—The growth is fusiform and a cloudiness radiates from the top of the canal into the medium; when magnified 10 times, delicate hairs are suggested. The cloudiness hangs downwards as a veil from the margin of the buff-coloured sunken nail-head. Liquefaction slowly takes place at first in a stratiform, then a funicular manner. There is formed an orange-coloured film and a precipitate which changes from white to orange.

Glucose-gelatine.—There is no gas produced.

Bouillon.—The medium becomes uniformly turbid and there is formed a whitish precipitate. The indol reaction is decided.

Potato.—There is formed a moist glistening, old-gold expansion.

Milk.—The casein is not coagulated and the reaction is unaltered.

The organism appears to be a subspecies of *Bac. arborescens*, Frankland, from which it differs in the appearance and colour on agar stroke. Compared with the organism which I have described under that name, the colonies on gelatine are white and the processes forming the mycelioid structure are more delicate and straight.

Bacillus stellatus, n.sp.

Shape, etc.—A stout rod of variable length and breadth, measures generally 0·8-1·0 : 2-3 μ; the ends are rounded. It is non-motile and is stained by Gram's method. It forms oval central spores quickly upon potato but slowly upon agar.

Agar plate.—The colonies are dull white, circular, raised and contoured. When magnified the structure appears folded and filamentous, the margin lobed and the edge smooth. The deep colonies are irregular, opaque and warty.

Agar stroke.—The growth is dry, glistening, luxuriant, greyish-white and longitudinally ribbed or terraced, the edge is straight and rough.

Gelatine plate.—The colonies at first are white and stellate, that is, straight processes radiate from a central point. When magnified, the surface and deep colonies are seen to have an irregular centre from which thin and thick straight processes radiate. The gelatine soon begins to liquefy and the processes become coarser and flocose. When the liquefaction has become circular, the contents are seen to be flocose and tufted, while from the margin radiating flocose strands penetrate into the softening gelatine.

Gelatine stab.—The filiform growth gives place to a funicular liquefaction which is very turbid. There is a precipitate of white floccules, but no film.

Glucose-gelatine.—No gas is produced.

Bouillon.—The medium becomes turbid; there is no film, the precipitate is white, flocose and filamentous. A slight indol reaction was obtained.

Nitrate-bouillon.—Nitrates are not reduced.

Milk.—The casein and the reaction are unaltered.

Potato.—The growth is white, dry, greasy, irregularly spreading and slightly pitted. The colour changes to a light stone or light drab.

This appears to be an ally of *Bac. verticillatus*, Rav., from which it appears to differ in the mycelioid or stellate appearance of young colonies in gelatine, the rapidity with which it forms spores on potato (2 days), the absence of a film on bouillon, and of a pellicle on the gelatine colonies, as well as in not changing the reaction of milk and in not affecting the colour of the medium in agar culture.

Two yeasts were separated, one of them pink with round to oval cells, the other a non-sporulating torula which forms in glucose-yeast-water a clear medium, with a white sediment and strong, thick pitted film. It may be Torula ii., Hansen. With the exception of *Bact. œrofaciens* and *Bact. gasoformans*, no gas was produced in lactose-gelatine by the organisms which have been described. Since these would have produced gas in gelatine in the absence of lactose, it may be said that none of these organisms produce gas from lactose.

Many of the bacteria in the Sydney water have points wherein they differ from what are probably the same organisms found in other parts of the world. Whether these differences are sufficient to warrant the formation of a new species is a matter of opinion. I consider the characters in which they differ to be practically fixed, since the cultures were made from about the fourth crop, and when the organism was diagnosed any difference from the described type was verified by a culture taken from what would be the sixth crop. For all practical purposes the types may be taken as fixed, since any work with Sydney water, sewage, etc., would be made upon cultures which had been grown in a similar manner and for no longer a time. The bacteriologist generally does not keep an unknown organism growing for several months before making a diagnosis. The characters may alter slowly after

a year's artificial culture, but that is only to be expected. Any rapid change in cultural character would have occurred before the fourth crop. This was noted in a previous paper to be the case with *Bac. piscicidus bipolaris*, the colonies of which in gelatine differed greatly in the second and fourth crops. From the fourth onwards, the type was fixed. Whether the differences between the types of other authors and these organisms are sufficient to warrant the formation of a new species, a new subspecies, or merely to note the difference, is a matter of opinion, and I leave it to other bacteriologists to accept my ruling or otherwise as they think fit. I believe the formation of subspecies is to be recommended, but for this the differences must not be vital, nor yet must they be insignificant so far as the diagnostic characters are concerned. In this a hard and fast line cannot be drawn, since a character which helps the diagnosis of one class of organism may be useless for another class. As an example, the liquefaction of gelatine may be cited. This action is of immense help to the bacteriologist and is used to distinguish classes of bacteria, yet it is too well known that the power may be gained or lost under laboratory conditions, and if this is the case a similar change may be expected to occur under natural conditions.

The organisms which have been described were isolated upon Abba's gelatine or dextrin-meat-agar. There are other media, however, less commonly employed in bacteriology and used for the purpose of suppressing such as have been obtained and permitting the growth of others. In a future paper I hope to describe the less commonly occurring bacteria isolated by the employment of selective methods.